SOIL-TRANSMITTED HELMINTH ENVIRONMENTAL SURVEILLANCE: FINAL PRESENTATION

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STRATEGIC ANALYSIS, RESEARCH & TRAINING CENTER
Department of Global Health | University of Washington

AGENDA

- Introductions
- Project Overview
- Literature Review
- SWOT Analysis
- Question and Answer

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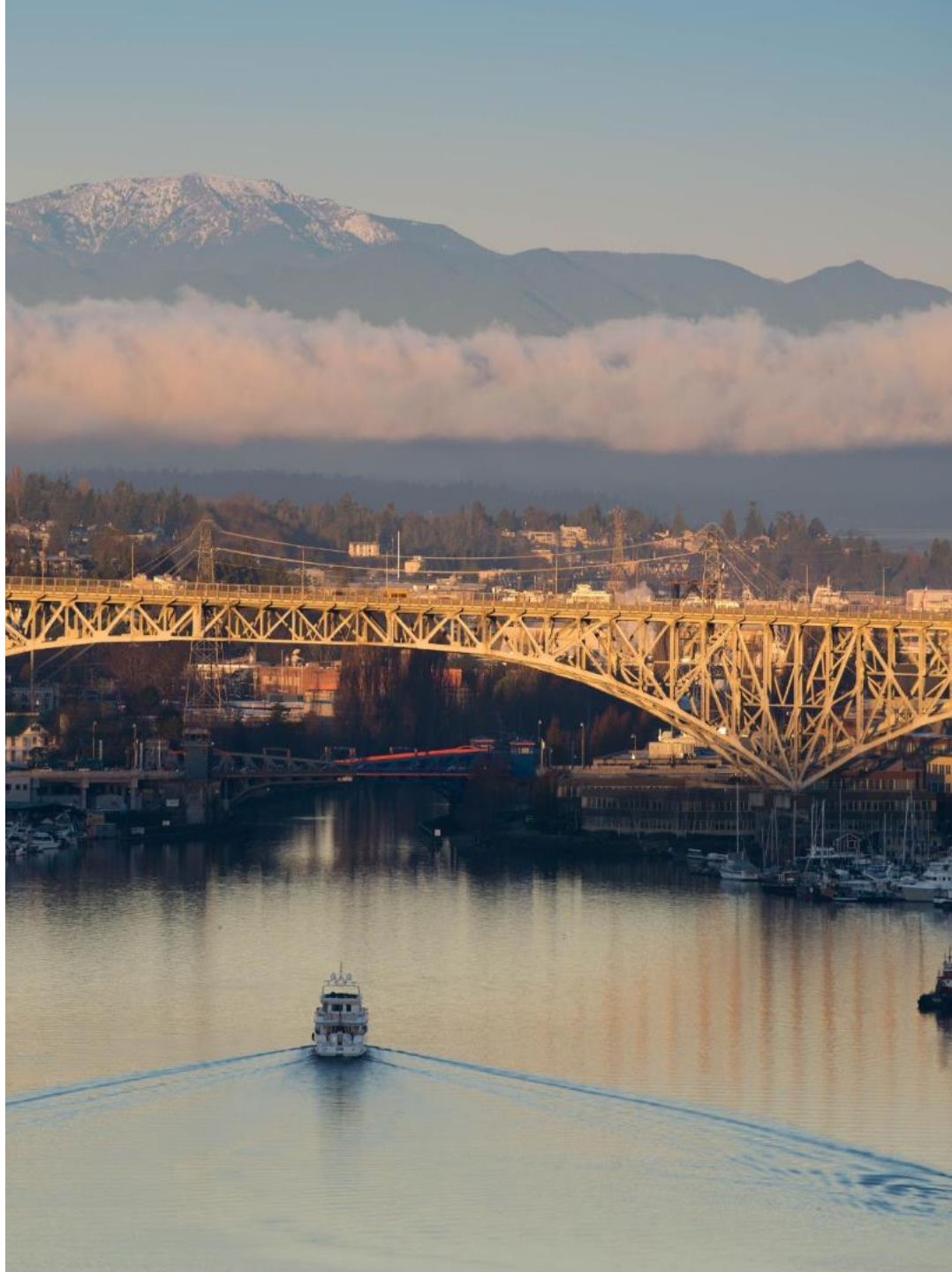

PROJECT TEAM

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START OVERVIEW

Leverages leading content expertise from across the University of Washington

Provides high quality research and analytic support to the Bill & Melinda Gates Foundation and global and public health decision-makers

Provides structured mentorship and training to University of Washington graduate research assistants

PROJECT OVERVIEW

BACKGROUND

Soil-Transmitted Helminths

- Group of Neglected Tropical Diseases that include Ascaris (*Ascaris lumbricoides)*, whipworm (*Trichuris trichiura*), and hookworm (*Anclostoma duodenale* and *Necator americanus*)
- Heavy infections produce symptoms including anemia, physical or cognitive growth stunting, abdominal pain
- Estimated global burden of disease 1.92 million DALYs (DALYs and HALE Collaborators 2017)

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BACKGROUND

Soil-Transmitted Helminths

- Primary diagnostic approach uses Kato-Katz technique for fecal sample analysis
  - Poor performance in low prevalence areas
  - Storage concerns (Bosch 2021)
  - Application to individual samples only
- Need exists for scalable environmental detection of helminth larvae in wastewater
  - Proof of Concept for urban sewer surveillance ongoing in India

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DELIVERABLES

1. Compiled database of results from PubMed and "Snowball" Search literature review, including abstracts and links to full text articles where available. Will inform ongoing efforts by the India Country Office to test proof of concept study in urban sewage surveillance.

2. Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis that includes qualitative indicators of scale-up potential for candidate STH wastewater surveillance technologies.

LITERATURE REVIEW

SEARCH STRATEGY

Database	Search Term Buckets*	Results Returned	Relevant Papers
PubMed	Soil Transmitted Helminths Sewage, wastewater Environmental Surveillance	83	**21**
Embase	Soil Transmitted Helminths Sewage, wastewater Environmental Surveillance	10	**3**
“Snowball” Search	--	--	**3**

* See appendix for full list of search terms

REVIEW PAPERS

Maya, 2006: *Comparison of Techniques for the Detection of Helminth Ova in Drinking Water and Wastewater*

- Focuses on isolation techniques
- Techniques Included:
  - US EPA
  - Membrane Filter
  - Leeds I
  - Faust

Table 10- Evaluation of techniques for detection of helminth ova

Analysis	Unit	Parameter	
Sensitivity	%	Recovery efficiency	Average Rating
Discrimination between close values	%	Interval of discrimination	5 AO/L at low concentrations Rating
	%		10 AO/L at high concentrations Rating
Precision	±	Standard deviation	5 AO/L Rating
			40 AO/L Rating
Recovery	%	Efficiency	Rating
Costs	US$		High TSS Rating
			Low TSS Rating
Personnel		Required level of training	High TSS Rating
			Low TSS Rating

REVIEW PAPERS

Ravindran, 2019: *A Review on the Current Knowledge and Prospects for the Development of Improved Detection Methods for Soil-Transmitted Helminth Ova for the Safe Reuse of Wastewater and Mitigation of Public Health Risks*

- Focuses on recovery and detection methods
- Techniques Included:
  - Recovery: sampling of wastewater/sludge, separation from solid matrix, filtration, sedimentation, flotation, phase extraction, factors influencing ova recovery
  - Detection methods: microscopy, PCR-based, Flow Cytometry, Digital PCR, Aptamers, Gold Nanoparticle-Based Colorimetric Biosensors, Surface Enhanced Raman Scattering (SERS), Smartphone-Based Detection, Isothermal Amplification Assays, Paper-Based Sensors

Table 5 - Advantages and drawbacks of currently available methods to enumerate and quantify helminth ova

Methods	Advantages	Limitations
Optical microscopy	Viability possible Cost-effective Require less lab space Stains can differentiate viable and non-viable ova	Time-consuming Less sensitivity and specificity Possible false positive results in the determination of viability using stain-based methods Species differentiation is not possible
PCR-based	Fast, specific and sensitive Multiplex PCR is possible Quantitative detection (qPCR) of target pathogen is rapid	Not possible to distinguish viable and dead ovaneed for well-equipped laboratory Multiple primers required Requirement of skilled personnel
Flow cytometry	Accurate and reliable Differentiate cells based on complexity	Particle size detection limit ranging between 3 μm and 20 μm Expensive and require skilled personnel

REVIEW PAPERS

Amoah, 2017: *Detection and quantification of soil-transmitted helminths in environmental samples: A review of current state-of-the-art and future perspectives*

- Focuses on isolation techniques, identification, viability determination, and emerging methods
- Techniques Included:
  - Egg recovery: separation, filtration, sedimentation, flotation, phase extraction
  - Viability determination: BacLight Dead/Live method
  - Nucleic acid based techniques: PCR, LAMP
  - Emerging techniques: Digital PCR, Image analysis software, Flow cytometry

Table 7 - Comparison of different techniques for detection of STHs in environment

Methodological category	Techniques	Advantages	Disadvantages
Conventional Techniques	WHO, USEPA, AMBIC, TULANE and several other variations of these methods	Easy to use, does not require sophisticated equipment hence less expensive.	Time consuming and laborious. Leaves too much for errors
Molecular Techniques	PCR, qPCR, LAMP	Rapid, quantitative (for qPCR), specific to species level identification	Expensive and requires skilled personnel
Emerging techniques	digital PCR	More specific, quantitative, no need of reference standards as is the case in other PCR techniques	Expensive and requires skilled personnel
	BacLight assay	Microscopic method to detect viability of STHs eggs	Expensive and requires skilled personnel

DELIVERABLE #1 - LITERATURE DATABASE

A	B	C	D	E	F
Study	Title	Relevance	Geography	Sample source	Sampling strategy
Jiménez, 2020	Helminth Egg Automatic Detector (HEAD): Improvements in development	Yes	Mexico, Costa Rica, Ecuac	* A new processing system for	NA
Amoah, 2018	Removal of helminth eggs by centralized and decentralized wastewater tre	Yes	eThekwini Municipality of K	WWAT and DEWAT plants	Samples were taken from the
Gyawali, 2016	Quantitative detection of viable helminth ova from raw wastewater, human	Yes	Australia and East Timor	Dog fecal samples from the sch	Flotation method described in
Ajonina, 2015	Microbial Pathogens in Wastewater Treatment Plants (WWTP) in Hamburg	Yes	Hamburg, Germany	WWTP in Hamburg	Wastewater collection from inf
Jaromin-Gleń, 201	Division of methods for counting helminths' eggs and the problem of efficie	Yes	NA	NA	Comparison of Multiple Strate
Verbyla, 2013	Taenia eggs in a stabilization pond system with poor hydraulics: concern fo	Yes	Yungas, Bolivia	The influent and effluent of the	Wastewater collection from inf
Bonatti T.R., 2016	Real scale environmental monitoring of zoonotic protozoa and helminth eg	Yes	Brazil	Biosolids collected at Opersan	Biosolid compost piles produc
Bastos VK, 2013	Detection and quantification of viable Ascaris sp. and other helminth eggs	Yes	Brazil	Monthly sewage sludge samplii	Activated sludge and sludge d
Dąbrowska J, 201	Assessment of viability of the nematode eggs (Ascaris, Toxocara, Trichuris	Yes	Poland	Sewage sludge from five munic	Combination of flotation and s
Gyawali P., 2018	Infectious helminth ova in wastewater and sludge: A review on public healt	Yes	NA	MISC. Review of currently publ	NA
Gyawali P, 2017	Quantification of hookworm ova from wastewater matrices using quantitati	Yes	NA	NA	Fresh dog faecal samples wer
Sengupta ME, 20	Use of Moringa oleifera seed extracts to reduce helminth egg numbers and	Maybe	Ghana	Water samples (irrigation water	NA
Navarro I, 2009	Application of Helminth ova infection dose curve to estimate the risks asso	No	Mexico	three previous studies: 1. estab	NA
Erlanger TE, 2007	Baseline health situation of communities affected by the Nam Theun 2 hyd	No	Lao PDR (Laos)	Persons in proximity to Nam Th	Large scale survey by NTPC,
Santander R, 2019	Development of a viability digital PCR protocol for the selective detection a	No	NA	E. amylovora-free plant materia	1-year-old apple 'Honeycrisp'
Pecson B, 2006	A real-time PCR method for quantifying viable ascaris eggs using the first i	Yes	NA	Ascaris suum eggs were purch	Genomic DNA and RNA were
Raynal M, 2012	Enumeration of viable and non-viable larvated Ascaris eggs with quantitati	Yes	NA	Ascaris suum eggs were purch	NA
Amoah I, 2017	Detection and quantification of soil-transmitted helminths in environmental	Yes	NA	NA	NA
Collender, P, 2015	Methods for Quantification of Soil-Transmitted Helminths in Environmental	Yes	NA	NA	Paper is a review of methods,
Rocha, M., 2016	Quantification of viable helminth eggs in samples of sewage sludge	Yes	NA	NA	USEPA; "amount of sludge tha
Ravindran, V. 202	Detection of Helminth Ova in Wastewater Using Recombinase Polymerase	Yes	NA	NA	Ascaris ova were obtained fro
Ravindran, V. 2019	A Review on the Current Knowledge and Prospects for the Development o	Yes	NA	NA	Based on WHO guidelines of
Maya, 2006	Comparison of techniques for the detection of helminth ova in drinking wat	Yes	NA	NA	NA

Link to Database

SWOT ANALYSIS

SWOT CRITERIA & DEFINITIONS

SWOT Field	Working Definition
Strengths	Characteristics of the candidate technique that lend themselves to use for STH Environmental Surveillance.
Weaknesses	Characteristics of the candidate technique that detract from their value for STH Environmental Surveillance.
Opportunities	How the strengths of the technique translate to implementation benefits in the context of global surveillance considerations.
Threats	How the weaknesses of the technique translate to implementation roadblocks in the context of global surveillance considerations.

SWOT CRITERIA & DEFINITIONS

Category	Definition	Low Score
Technical Feasibility	Does proof of concept for this technique already exist?	This technique is hypothetical or as-of-yet undeveloped.
Operational Feasibility	If this technique were technically feasible, would operating it at scale prove particularly difficult at the program level?	Significant technical or logistical barriers to scale exist with no clear roadmap to overcome. Much specialized training or equipment would be required to implement.
Integration	How easily could this technique be integrated into existing surveillance systems (i.e. typhoid, SARS)?	This technique is not compatible with the technology or systems in existing surveillance.
Cost	Will operational or development costs likely be a major hurdle relative to other surveillance techniques?	Significant funding will be required to develop the materials needed for this technique, or cost per use will be much higher than competing techniques.

Low | Medium | High

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SWOT CRITERIA & DEFINITIONS

Category	Definition	Medium Score
Technical Feasibility	Does proof of concept for this technique already exist?	Proof of concept for this technique has been demonstrated in laboratory settings, limited field use.
Operational Feasibility	If this technique were technically feasible, would operating it at scale prove particularly difficult at the program level?	Logistical barriers to scale may exist but these could be overcome with the investment of significant funding or attention.
Integration	How easily could this technique be integrated into existing surveillance systems (i.e. typhoid, SARS)?	This technique could be partially integrated with existing surveillance under the right circumstances.
Cost	Will operational or development costs likely be a major hurdle relative to other surveillance techniques?	Costs are likely to exceed existing technique costs but these may decrease over time or will be comparable to competing techniques.

Low | Medium | High

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SWOT CRITERIA & DEFINITIONS

Category	Definition	High Score
Technical Feasibility	Does proof of concept for this technique already exist?	This technique has been implemented in field settings with success.
Operational Feasibility	If this technique were technically feasible, would operating it at scale prove particularly difficult at the program level?	This technique could be scaled simply if sufficient resources were made available. Minimal specialized training or equipment required to implement.
Integration	How easily could this technique be integrated into existing surveillance systems (i.e. typhoid, SARS)?	This technique could be easily integrated with existing systems without requiring major changes.
Cost	Will operational or development costs likely be a major hurdle relative to other surveillance techniques?	Costs are likely to be lower than competing techniques.

Low	Medium	High

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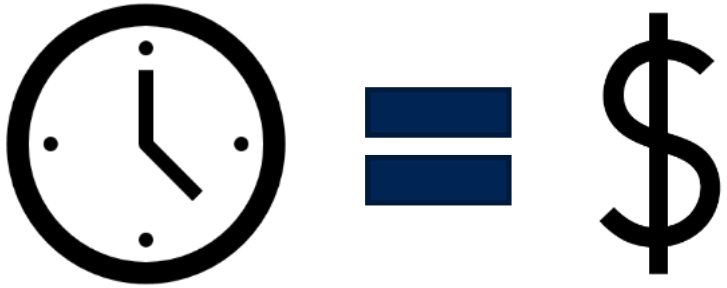
SWOT CRITERIA & DEFINITIONS

- Expert Opinion (Judd Walson)
- Cost determination will vary by sampling framework
  - Cost per use for PCR would be prohibitive if running individual samples for an MDA program, microscopy is much cheaper on a per use basis
  - Cost potentially becomes much more manageable in an environmental surveillance context
  - Running one PCR gel per sample site at a fixed interval may be cheaper than paying for microscopy technician to examine samples for 8 hours
- Cost per sample for emerging methods may be low once standardized, but up-front investment should be considered



SWOT – ISOLATION TECHNIQUES

Surveillance Techniques			
Isolation Methods	**Pros** Necessary method for many identification techniques, allows use from different sample types	**Cons** Additional step, may not be required for all identification methods (theoretical)	**US EPA**
			Leeds I
			Faust
			Membrane Filter

SWOT – ISOLATION OVERVIEW

Method	Description
US EPA	**Relies on flotation** (zinc sulfate*) **and diphasic sedimentation** to separate and concentrate ova. Sample volume is 5 L water for all solids concentrations.
Leeds I	Uses several **flotation** (zinc sulfate*) **and centrifugation** steps to separate and concentrate ova. Readings are taken on aliquots and extrapolated for final concentration. Sample volume is 1 L for water with high TSS; 40L for water with low TSS.
Faust	Uses several **flotation** (zinc sulfate*), **centrifugation, and sedimentation** steps to separate and concentrate ova. Readings are take on aliquots and extrapolated for final concentration. Sample volume is 1 L for water with high TSS; 40L for water with low TSS.
Membrane Filter	Utilizes 1 L of **water, zinc sulfate*, and flotation filtration**; recovers ova using a **cellulose acetate membrane.** Initially developed for use with protozoa.

* Methods evaluated using zinc sulfate in lab environment, but magnesium sulfate and sodium chloride are also used to adjust the specific gravity of solute.

SWOT – ISOLATION EXEMPLAR

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
US EPA	**Relies on flotation** (zinc sulfate, magnesium sulfate) **and diphasic sedimentation** to separate and concentrate ova. Sample volume is 5 L water for all solids concentrations.	* useful for samples with high and low solids concentration *recovers largest variety of helminth ova (based on specific gravity) * lowest overall cost (~$39.40 USD per sample) (Maya, 2006)	* some less dense ova may be outside the range of recovery * relies on overnight sedimentation, requiring more time than centrifugal techniques (Ravindran, 2019)	* technique performs well with varied samples (wastewater v drinking water) * lowest cost of isolation techniques (Maya, 2006) * reliance on passive sedimentation make this appropriate for resource limited settings (Ravindran, 2019	* Some ova may not float or sink in the appropriate phase, resulting in missed or underreported helminths * unrecovered ova may lead to underestimation (Ravindran, 2019)	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low	Medium	High

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Link to SWOT draft

SWOT – IDENTIFICATION TECHNIQUES

Surveillance Techniques			
Conventional Identification Methods	**Pros** Cheap, low capacity required, little equipment and space	**Cons** Poor sensitivity, differentiation between human and animal worms, slow	**Culture-Based**
			Vital Staining
			LIVE/DEAD Kit
Molecular Identification Methods			Polymerase Chain Reaction (PCR)
			Isothermal Amplification
			Real-Time Quantitative PCR (qPCR)
			Droplet Digital PCR (ddPCR)
Emerging Identification Methods			Aptamers
			Helminth Eggs Automatic Detector
			Flow Cytometry
			Gold Nanoparticle-based Calorimetric Biosensors
			Surface Enhanced Raman Scattering
			Smartphone-Based Detection
			Paper-Based Sensors

SWOT – CONVENTIONAL OVERVIEW

Method	Description
Culture-based (incubation and microscopy)	**Incubation** of isolated ova to **develop larvae and assess viability** using microscopy; incubation time varies from 21 - 30 days.
Vital Stains	**Identify** isolated ova and **assesses viability by selectively coloring dead cell walls and viewing under microscope.** Specific dyes include: Trypan Blue, Congo red, Eosin Y, Methyl green, Safranin O, etc.
BacLight LIVE/DEAD Kit	Assesses parasite egg viability through differences in **membrane integrity of viable and non-viable cells.** DNA-labelling dyes used; cells fluoresce green in viable eggs and red in non-viable eggs under microscope.

SWOT – CONVENTIONAL EXEMPLAR

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Culture-based (incubation and microscopy)	**Incubation** of isolated ova to develop larvae. **Identification and viability assessment using microscopy**; incubation time varies from 21 - 30 days.	* reliably determines viability 86% of time * most widely used viability technique (considered "gold standard") * captures ova (Ravindran, 2019)	* requires time (21-30 days) for maturation of helminth ova * contaminants may yield false positive result * enumeration of ova dependent on recovery method (Ravindran, 2019) *developmental stages may interfere with viability determination (Rocha, 2016)	* Provides accurate measurement of viability ova * Reduces human error from microscopy identification * little lab space, equipment, and reagent costs (Ravindran, 2019)	* lag time from sampling to determination of viability can take up to a month * identification and viability determination require extensive training * species determination may not be possible (Ravindran, 2019)	Technical Feasibility Operational Feasibility Integration Cost

Low	Medium	High

START CENTER

Link to SWOT draft

Surveillance Techniques			
Isolation Methods			US EPA
			Leeds I
			Faust
			Membrane Filter
Conventional Identification Methods			Culture-Based
			Vital Staining
			LIVE/DEAD Kit
Molecular Identification Methods	**Pros** Fast, highly sensitive, multiplexing	**Cons** Expensive, viability determination	**Polymerase Chain Reaction (PCR)**
			Isothermal Amplification
			Real-Time Quantitative PCR (qPCR)
			Droplet Digital PCR (ddPCR)
Emerging Identification Methods			Aptamers
			Helminth Eggs Automatic Detector
			Flow Cytometry
			Gold Nanoparticle-based Calorimetric Biosensors
			Surface Enhanced Raman Scattering
			Smartphone-Based Detection
			Paper-Based Sensors

SWOT – MOLECULAR OVERVIEW

Technique	Description
Polymerase Chain Reaction (PCR)	* Nucleic acid amplification technique in wide use throughout the world. Requires laboratory equipment for cycles of heating and cooling during enzyme activity.
Isothermal Amplification	* Analogous to PCR amplification of DNA but without thermocycling requirements. * Loop Mediated Isothermal Amplification and Recombinase Polymerase Amplification are two leading techniques
Real-Time Quantitative PCR (qPCR)	* PCR variant combined with standard curve generation to provide quantification.
Droplet Digital PCR (ddPCR)	* PCR variant that utilizes microwells to split the samples into several partitions in nanoliter to provide absolute quantification.

SWOT – MOLECULAR EXEMPLAR

Technique	Description	Strengths	Weaknesses	Opportunities	Threats	Color Coding
Isothermal Amplification Assays	* Analogous to PCR amplification of DNA but without thermocycling requirements. *Loop Mediated Isothermal Amplification and Recombinase Polymerase Amplification are two leading techniques	* Extremely sensitive and specific, lower limit of detection at one ovum (Ravindran , 2019) * Easier to perform in field settings than traditional PCR, no thermocycling requirements * Fast and visual	* Currently no guaranteed LAMP multiplex option as for qPCR (PoC for RPA multiplexing and wastewater detection) * LAMP for STH done on fecal samples, not wastewater (Rashwan 2017) * Complex primer design step * High false positive rate for LAMP (Ravindran 2019)	* LAMP and RPA can be performed in low resource settings and with less equipment and training than PCR * RPA used with multiplex LFA strips (Ravindran 2020) * Low per sample cost, high speed, and ease of operation would allow for extensive field use	* Turbidity variance in wastewater not yet tested * Demand for LFAs may create supply issue competing with Malaria, COVID, Pregnancy tests, etc.	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low | Medium | High

START CENTER

Link to SWOT draft

Surveillance Techniques			
Isolation Methods			US EPA
			Leeds I
			Faust
			Membrane Filter
Conventional Identification Methods			Culture-Based
			Vital Staining
			LIVE/DEAD Kit
Molecular Identification Methods			Polymerase Chain Reaction (PCR)
			Isothermal Amplification
			Real-Time Quantitative PCR (qPCR)
			Droplet Digital PCR (ddPCR)
Emerging Identification Methods	**Pros** High throughput, high sensitivity	**Cons** Most methods lack field testing, expensive	**Aptamers**
			Helminth Eggs Automatic Detector
			Flow Cytometry
			Gold Nanoparticle-based Calorimetric Biosensors
			Surface Enhanced Raman Scattering
			Smartphone-Based Detection

SWOT – EMERGING OVERVIEW

Technique	Description
Aptamers	Either single stranded **RNA or DNA molecules** that bind **surface receptors**. Used for differentiating tissues, viruses and bacteria. Potential use in STH.
Helminth Eggs Automatic Detector, HEAD	Automated analysis of ***light microscopy images*** incorporating various ***image processing algorithms*** for quantification of pathogenic helminth eggs of global medical importance.
Flow Cytometry	Analysis of multiple ***physical properties*** of eggs/cysts, as they ***flow*** in a fluid stream through a ***beam of light*** for the differentiation of one cell from the other.
Gold Nanoparticle-Based Colorimetric Biosensors	Use of ***light*** and ***gold-based sensors*** for detecting and differentiating STHs ova based on the differences in their surface moieties.
Surface Enhanced Raman Scattering (SERS)	Detection of ***in-situ biosynthesis of metal nanoparticles***. Has been used in detecting bacteria and seems to have potential for use STH ES.
Smartphone-Based Detection	***Lab-on-a-chip technology***, bringing together the ***high precision*** and ***sensitivity*** of diagnostic techniques with the ***connectivity*** and ***computational power*** of smartphones.

SWOT – EMERGING EXEMPLAR

Technique	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Helminth Eggs Automatic Detector, HEAD	* Automated analysis of **light microscopy images** incorporating various **image processing algorithms** for quantification of pathogenic helminth eggs of global medical importance. *(Collender 2015)* * Updated, now second version *(Jiménez 2020)*	* Uniform criteria for both **identification** and **quantification** * Reduced result **turn-around time** * Does not require highly trained personnel * **High sensitivity and specificity** in otherwise **difficult environmental samples:** oil, wastewater, biosolids, excreta, and sludge *(Jiménez 2020)*	* **Only** differentiates between fertile and infertile eggs for **Ascaris** but not the other helminths * **Misidentification:** Nuances in **developmental stages** may be missed without human eyes *(Jiménez 2020)*	* Automated image identification has the potential to rapidly **accelerate** and **standardize** quantification of STHs in environmental samples *(Collender 2015)*	* A **specialized microscope** and **camera** are required, with specific settings that are needed in order to produce an image of "**sufficient quality**." * The **cost** of the technology could limit **uptake** of the technique	**Technical feasibility** **Operational feasibility** **Integration** **Cost**

Low | Medium | High

START CENTER

Link to SWOT draft

DELIVERABLE #2 – SWOT ANALYSIS

Technique	Description	Strengths	Weaknesses	Opportunities	Threats	Color Coding
			Molecular Methods			
Polymerase Chain Reaction (PCR)	Amplification of target genetic sequences through cycles of heating and cooling and treatment with a variety of enzymes	* Well-documented method with consistent performance * Variants such as Reverse-Transcriptase PCR can be used in viability determinations	* Laboratory required for thermocycling steps * Technical training required * Lysing step needed for helminth ova due to tough outer tegument	PMA/Reverse-Transcriptase PCR may make this area the most sensitive and reliable diagnostic method for environmental samples (Ravindran 2019)	* Lysing and sonication or bead treatment needed to break down helminth coat may make cost and effort prohibitive for large numbers of samples (Ravindran 2019)	Technical Feasibility Operational Feasibility Integration Cost
Isothermal Amplification Assays (LAMP/RPA)	Analogous to PCR amplification of DNA but without thermocycling requirements. Can detect down to one ovum in a sample.	* Easier to perform in field settings than traditional PCR due to lack of thermocycling requirements * Fast and visual	(Rashwan 2017 DOI - 10.1186/s13071-017-2420-1) * Complex primer design step * High false positive rate for LAMP (Ravindran 2019)	(Ravindran 2020 DOI - 10.3390/w12030691) * Low per sample cost, high speed, and ease of operation allow for extensive field use	* Turbidity variance in wastewater not yet tested * Demand for LFAs may create supply issue competing with Malaria, COVID, Pregnancy tests	Technical Feasibility Operational Feasibility Integration Cost
Real-Time Quantitative PCR, qPCR	PCR variant combined with standard curve generation to provide quantification.	combined with PMA (Ravindran 2019) * Quick processing time * Allows for absolute quantification of target analyte	* Susceptible to impurity and amplification errors (Ravindran 2019) * Higher per-sample cost than traditional PCR	* Classifies viability and quantity * Could be adapted for health risk assessment	settings (best performance with fecal samples, worse perfromance with wastewater and soil samples) * Standard curve calculations may limit use to centers with highly trained staff	Technical Feasibility Operational Feasibility Integration Cost
Droplet Digital PCR, ddPCR	technique utilizes microwells that can split the samples into several partitions in nanoliter to provide absolute quantification.	low-copy-number Variants (Kuypers 2017) * Can be used in detection and absolute quantification	2019 conflicts with description as cheaper in Rajapaksha, 2019) * More complex to perform, lower throughput vs PCR (Kuypers 2017)	thresholds * Commercial kits already exist * Automated nature supports routine surveillance	low-resource settings * Low throughput and limited multiplexing may hamper surveillance capabilities	Technical Feasibility Operational Feasibility Integration Cost
			Emerging Methods			
Aptamers	and have the ability to differentiate proteins that are homologous and possess changes only in a few amino acids.	* In theory, possible to create for any desired organism * High specificity and high affinity (Ravindran, 2019) * Once found, simple to generate	* Intensive discovery process required * No helminth method yet and low hit rates for detecting new candidates (Zhuo, 2017)	* Proof of concept for Schistosoma japonicum (detection ratio 80.5% in Long 2016)	in-vivo * No indication of species differentiation and viability determination * No prior surveillance use	Technical Feasibility Operational Feasibility Integration Cost
Helminth Eggs Automatic Detector, HEAD	quantification of pathogenic helminth eggs of global medical importance. (Collender 2015)	wastewater, oil, biosolids, excreta, and sludge with high sensitivity and specificity (Jiménez 2020)	* Only differentiates between fertile and infertile eggs for Ascaris but not the other helminths (Jiménez 2020)	and standardize quantification of STHs in environmental samples (Collender 2015)	lack of any of those is a potential thread. * The cost of the technology could be limiting utility of the technique	Technical Feasibility Operational Feasibility Integration Cost
Flow cytometry	particle or cell from 0.2–150 micrometers in size is suitable for analysis (Vesey et al., 1997).	been combined with real-time PCR and fluorescent biosensors to achieve more accurate results (Ravindran 2019)	ranging between 3 µm and 20 µm * Expensive and require skilled personnel (Ravindran 2019)	dyes to differentiate non-/viable eggs (e.g., BacLight Live/Dead staining to determine STH eggs' viability) (Amoah 2017)	* The complex matrix of wastewater and sludge result in the possibility of clogging the machine (Amoah 2017)	Technical Feasibility Operational Feasibility Integration Cost
Gold Nanoparticle-Based Colorimetric Biosensors	differentiate STHs ova based on the difference in their surface moieties. (Ravindran 2019)	* No sophisticated instrumentation is required (Aldewachi 2017 - DOI: 10.1039/c7nr06367a)	* Low sensitivity and long run-time for traditional approaches, LFA techniques still in development (Aldewachi 2017)	needs further innovation and validation. (Ravindran 2019)	development. Reusability protocols non-existent currently. (Aldewachi 2017 - DOI: 10.1039/c7nr06367a)	Technical Feasibility Operational Feasibility Integration Cost

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Link to SWOT Analysis

SWOT – QUALITATIVE OVERVIEW

Surveillance Techniques		Technical Feasibility	Operational Feasibility	Integration	Cost
Isolation Methods	US EPA				
	Leeds I				
	Faust				
	Membrane Filter				
Conventional Identification Methods	Culture-Based				
	Vital Staining				
	LIVE/DEAD Kit				
Molecular Identification Methods	Polymerase Chain Reaction (PCR)				
	Isothermal Amplification				
	Real-Time Quantitative PCR (qPCR)				
	Droplet Digital PCR (ddPCR)				
Emerging Identification Methods	Aptamers				
	Helminth Eggs Automatic Detector				
	Flow Cytometry				
	Gold Nanoparticle-based Calorimetric Biosensors				
	Surface Enhanced Raman Scattering				
	Smartphone-Based Detection				
	Paper-Based Sensors				

Low | Medium | High

LIMITATIONS

- Color coding designations present ***qualitative indicators*** of implementation considerations, not exact representations

- Many of these techniques would require additional ***field studies*** to determine the validity of assumptions related to scale-up and implementation

CONCLUSIONS

- Expert opinion (Scott Meschke)
  - **Molecular techniques** have the **strongest future potential**
  - A **critical overall threat**: Lack of clear link to **action thresholds**

- Isothermal molecular techniques performed best overall in our qualitative assessment
  - Biggest concern for molecular techniques was **cost**
  - Cost considerations **may be mitigated** depending on use case

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QUESTIONS?

THANK YOU

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STRATEGIC ANALYSIS,
RESEARCH & TRAINING CENTER

APPENDIX

SEARCH TERMS

Database	Search Terms	Relevant Papers	Results Returned
PubMed	(“Soil Transmitted Helminth*” OR “Helminths” [MeSH] OR “Ascaris” [MeSH] OR “Ascaris lumbricoides” [MeSH] OR “hookworm” [All Fields] OR “whipworm” [All Fields] OR “Trichuris trichiura” [All Fields] OR “roundworm” [All Fields] OR “Ancylostoma duodenale” OR Necator americanus [MeSH]) **AND** ((“waste water” [MeSH] OR “Sanitation” [MeSH] OR “Sanitary Engineering” [MeSH] OR “Water Purification” [MeSH] OR “sewage” [MeSH] OR sludge OR “Waste Management” [MeSH] OR “Toilet Facilities” [MeSH] OR “Waste Disposal Facilities” [MeSH]OR "Toilet Facilities"[MeSH]) **AND** (“environmental monitoring" [MeSH] OR “Environmental surveillance” OR “Epidemiological Monitoring” [MeSH])) **AND** English[Language]	**21**	83
Embase	('soil transmitted helminth'/exp OR 'soil transmitted helminth' OR 'soil transmitted helminthiasis'/exp OR 'soil transmitted helminthiasis' OR 'ascaris'/exp OR 'ascaris' OR 'ascaris lumbricoides'/exp OR 'ascaris lumbricoides' OR 'hookworm'/exp OR 'hookworm' OR 'trichuris trichiura'/exp OR 'trichuris trichiura' OR 'ancylostoma duodenale'/exp OR 'ancylostoma duodenale' OR 'necator americanus'/exp OR 'necator americanus’) **AND** ('wastewater'/exp OR 'wastewater' OR 'municipal wastewater'/exp OR 'municipal wastewater' OR 'liquid waste'/exp OR 'liquid waste' OR 'sewage'/exp OR 'sewage' OR 'sludge'/exp OR 'sludge' OR 'waste management'/exp OR 'waste management' OR 'sanitation'/exp OR 'sanitation' OR 'water management'/exp OR 'water management’) **AND** ('sanitary surveillance'/exp OR 'sanitary surveillance' OR 'wastewater-based epidemiology'/exp OR 'wastewater-based epidemiology' OR 'environmental surveillance'/exp OR 'environmental surveillance' OR 'environmental monitoring'/exp OR 'environmental monitoring’) **AND** [embase]/lim **AND** [english]/lim	**3**	10

Surveillance Techniques	
Isolation Methods	US EPA
	Leeds I
	Faust
	Membrane Filter
Conventional Identification Methods	Culture-Based
	Vital Staining
	LIVE/DEAD Kit
Molecular Identification Methods	Polymerase Chain Reaction (PCR)
	Isothermal Amplification
	Real-Time Quantitative PCR (qPCR)
	Droplet Digital PCR (ddPCR)
Emerging Identification Methods	Aptamers
	Helminth Eggs Automatic Detector
	Flow Cytometry
	Gold Nanoparticle-based Calorimetric Biosensors
	Surface Enhanced Raman Scattering
	Smartphone-Based Detection
	Paper-Based Sensors

SWOT – ISOLATION METHODS (1)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Leeds I	Uses flotation (zinc sulfate) and centrifugation to separate and concentrate ova.Sample volume is 1 L for water with high TSS; 40 for water with low TSS.	* only one aliquot required for analysis * method is most precise, when compared to similar technique (Maya, 2006)	* has challenges with detecting small numbers of parasites * egg wall may collapse during centrifugation * highest cost method of isolation techniques * requires differing volumes for high and low solid concentrations (Maya, 2006)	* allows extrapolation of ova concentration with small sample volume * provides precise isolation of ova (Maya, 2006)	* technique may not provide accurate results with microscopy or with small numbers of parasites * requires centrifuge for technique; may not be appropriate for all settings * Challenging to identify helminths using microscopy if cell walls have collapsed, resulting in missed helminths (Maya, 2006)	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low	Medium	High

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Link to SWOT draft

SWOT – ISOLATION METHODS (2)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Faust	Sample volume is 1 L for water with high TSS; 40 for water with low TSS; uses flotation (zinc sulfate), centrifugation, and additional sedimentation step.	* only one aliquot required for analysis * relatively low cost for materials and human resources (~$42.40 USD per sample) (Maya, 2006)	* has challenges with detecting small numbers of parasites * egg wall may collapse during centrifugation * extrapolated concentrations from aliquots of sample provide high estimates * requires differing volumes for high and low solid concentrations (Maya, 2006)	* allows extrapolation of ova concentration with small sample volume * low costs for equipment and implementation (additional expenses include extensive training); ease of scalability (Maya, 2006)	* requires centrifuge for technique; may not be appropriate for all settings * technique may not provide accurate results with microscopy or with small numbers of parasites * Challenging to identify helminths using microscopy if cell walls have collapsed, resulting in missed helminths (Maya, 2006)	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low | Medium | High

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Link to SWOT draft

SWOT – ISOLATION METHODS (3)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Membrane filter	Utilizes 1 L of water, zinc sulfate, and flotation filtration; recovers ova using a cellulose acetate membrane. Initially developed for use with protozoa.	* high rate of recovery for eggs in water with low solid concentration * second lowest degree of training required (behind USEPA technique) * sieve has pore size of 20 micrometers, smaller than most helminth ova * relatively low cost (~$40.67 USD per sample) (Maya, 2006)	* not appropriate for all sample types; solids become an issue in wastewater samples (Maya, 2006)	* very sensitive and efficient * suitable in low resource settings (Maya, 2006)	* appropriate with limited samples; not suited for wastewater or reclaimed water (Maya, 2006)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

START CENTER

Link to SWOT draft

SWOT – CONVENTIONAL METHODS (1)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Vital Staining	Assesses parasite viability by selectively coloring dead cell walls. Specific dyes include: Trypan Blue, Congo red, Eosin Y, Methyl green, Safranin O, etc.	* Short turnaround time for determination * Minimal equipment required * Able to assess viability * less steps for staining when compared to LIVE/DEAD method (Gyawali, 2018)	* 39% viability determination * Only stains dead cells * Prone to misidentification or inaccurate staining * sensitivity is limited by threshold of microscpe (Gyawali, 2018)	* Low start up costs * Classifies viability of eggs, providing more insight into extent of outbreak potential * Can be performed in low resource settings (Gyawali, 2018)	* Challenging to implement at large scale * May require extensive training of staff * may have inaccurate staining, leading to misclassification of viability * low reliability of viability determination (Gyawali, 2018)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – CONVENTIONAL METHODS (2)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
LIVE/DEAD Kit	Assesses parasite egg viability through differences in membrane integrity of viable and non-viable cells. DNA-labelling dyes used; cells fluoresce green in viable eggs and red in non-viable eggs.	*Capacity to assess helminth viability * Minimal equipment required * 78%-85% viability detection * Short turn around time for determination * does not cause damage to the viability of ova (Gyawali, 2018)	* Further classification of helminths by sight is error-prone *ova may be inactivated by staining chemicals * may have difficulty determining results from indiscriminate binding of stains (Gyawali, 2018)	* Low start up costs * Classifies viability of eggs, providing more insight into extent of outbreak potential * Can be performed in low resource settings (Gyawali, 2018)	* Challenging to implement at large scale * May require extensive training of staff * may have inaccurate staining, leading to misclassification of viability (Gyawali, 2018)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – MOLECULAR METHODS (1)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Polymerase Chain Reaction (PCR)	Amplification of target genetic sequences through cycles of heating and cooling and treatment with a variety of enzymes.	* Highly sensitive and specific * Well-documented method with consistent performance * Variants such as Reverse-Transcriptase PCR can be used in viability determinations	* High cost per sample to run * Laboratory equipment required for thermocycling steps * Technical training required * Lysing or beating step needed for helminth ova due to tough outer tegument	* Advancements in viability quantification through PMA/Reverse-Transcriptase PCR may make this area the most sensitive and reliable diagnostic method for environmental samples (Ravindran 2019)	* Lysing and sonication or bead treatment needed to break down helminth coat may make cost and effort prohibitive for large numbers of samples (Ravindran 2019)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – MOLECULAR METHODS (2)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Real-Time Quantitative PCR, qPCR	PCR variant combined with standard curve generation to provide quantification.	* Able to differentiate between viable and non-viable ova when combined with PMA (Ravindran 2019) * Quick processing time * Allows for absolute quantification of target analyte	* Requires extraction of ova via flotation * Requires standard curves for quantification * Susceptible to impurity and amplification errors	* Scalable method * Classifies viability of ova * Could be adapted for health risk assessment	* May not be appropriate for all settings (best performance with fecal samples, worse perfromance with wastewater and soil samples) * Standard curve calculations may limit use to centers with highly trained staff	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – MOLECULAR METHODS (3)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Droplet Digital PCR, ddPCR	This version of the PCR technique utilizes microwells that can split the samples into several partitions in nanoliter to provide absolute quantification.	* Absolute quantification, no standard curve * Improved interlaboratory commutability * More precise than qPCR (Ravindran 2019) * Better detection of low-copy-number Variants (Kuypers 2017) * Can be used in detection and absolute quantification	* Less accurate quantification of larger amplicons than qPCR * Limited multiplexing exacerbated if assay requires internal control * More expensive instrumentation and reagents than qPCR (Ravindran 2019 conflicts with description as cheaper in Rajapaksha, 2019) * More complex to perform, lower throughput vs PCR (Kuypers 2017)	* Quantitative detection of pathogens provides link to action thresholds * Commercial kits already exist * Lack of standard curve reduces training needs * Automated nature supports routine surveillance	* High expense and required technical capacity likely prohibitive in low-resource settings * Low throughput and limited multiplexing may hamper surveillance capabilities	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – EMERGING METHODS (1)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Aptamers	Either single stranded **RNA or DNA molecules** that bind **surface receptors**. Used for differentiating tissues, viruses and bacteria. Potential use in STH.	In theory, possible to create for any desired organism, high specificity, high affinity (Ravindran, 2019), once found easy to generate	High cost, intensive discovery process required, no helminth method yet, low hit rates for detecting new candidates (Zhuo, 2017)	Proof of concept for Schistosoma japonicum (detection ratio 80.5% Long, 2016), helminth ova could be next	In-vitro aptamers not always effective in-vivo, no indication of species differentiation and viability determination, no prior surveillance use	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – EMERGING METHODS (2)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Flow cytometry	Flow cytometry simultaneously measures and analyzes multiple physical properties of eggs/cysts, as they flow in a fluid stream through a beam of light. Properties such as relative size, granularity or complexity and fluorescence intensity are used in the differentiation (Vesey et al., 1997).	* Accurate and reliable * Could be used in the detection and quantification and determining the viability of STH eggs * Differentiates eggs based on complexity * Recently, flow cytometry has been combined with real-time PCR and fluorescent biosensors to achieve more accurate results (Ravindran 2019)	* Particle size detection limit ranging between 3 µm and 20 µm * Expensive and require skilled personnel (Ravindran 2019)	* No report describing the method use in STH eggs detection (potential knowledge gap) * Potential to incorporate fluorescent dyes to differentiate non-/viable eggs (e.g., BacLight Live/Dead staining to determine STH eggs' viability) (Amoah 2017)	* The high cost hinders routine use especially in developing countries * The complex matrix of wastewater and sludge result in the possibility of clogging the machine (Amoah 2017)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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Link to SWOT draft

SWOT – EMERGING METHODS (3)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Gold Nanoparticle-Based Colorimetric Biosensors	* This is a technique that uses light and gold-based sensors can be applied to detect and differentiate STHs ova based on the difference in their surface moieties. (Ravindran 2019)	* A simple method that only require a few steps for the detection of target molecules. * No sophisticated instrumentation is required (Aldewachi 2017 - DOI: 10.1039/c7nr06367a)	* Very specific analyte binding properties required. Surface moieties of helminths PoC but not in use. * Comparatively high limit of detection at ~100 ova/Liter of wastewater (Ravindran 2019 - https://www.ncbi.nlm.nih.gov/pubmed/31080763) * Low sensitivity and long run-time for traditional approaches, LFA techniques still in development (Aldewachi 2017)	* Nanoparticles can be used in the development of biosensors and be incorporated into smart phones or portable devices for mobile sensing – needs further innovation and validation. (Ravindran 2019)	* Turbidity encountered in wastewater and sludge samples can lead to non-specific aggregation of AuNPs, thus triggering false positive results (Ravindran 2019) * Transforming these sensors into point of care devices awaits further development. Reusability protocols non-existent currently. (Aldewachi 2017 - DOI: 10.1039/c7nr06367a)	Technical Feasibility Operational Feasibility Integration Cost

Low | Medium | High

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SWOT – EMERGING METHODS (4)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Surface Enhanced Raman Scattering (SERS)	Surface-enhanced Raman scattering (SERS) is a technique for the detection of living bacteria in drinking water. The Raman signals intensity of bacteria after AgNP synthesis mainly depends on the zeta potential of the cell wall. (Zhou 2014)	* The utilization of synthesized metal nanoparticles enhanced the Raman signal of bacteria by 30-fold * Minimal processing time * Easier handling * Minimal reactant volumes * Less volume of the sample * Greater sensitivity * Greater selectivity (Ravindran 2019)	* The detection of chemical transformations that occur during in-situ biosynthesis of metal nanoparticles are quite challenging as it occurs at the interfaces. (Ravindran 2019) * Some challenges in detection depending on the tissue under study (Langer 2020 - https://doi.org/10.1021/acsnano.9b04224)	* SERS has been utilized in the detection of viable bacteria in drinking water * Potential to develop SERS-biosensors to differentiate species of STH ova [ultra-sensitive, rapid, and easy-to-use method of diagnosis] (Ravindran 2019)	* Feasibility: potential to fulfill the diagnostic requirements in endemic areas yet to be studied (Ravindran 2019) *Substrate reproducibility issue and SERS Intensity Fluctuations cast doubt on reliability of quantification results (Langer 2020)	Technical Feasibility Operational Feasibility Integration Cost

Low	Medium	High

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Link to SWOT draft

SWOT – EMERGING METHODS (5)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Smartphone-Based Detection	Smartphone-based imaging (biosensors and lab-on-a-chip) and sensing platforms are emerging as promising alternatives for decentralizing diagnostic tests offering practical features such as portability, cost-effectiveness and connectivity. (Hernández-Neuta 2019 - doi: 10.1111/joim.12820)	* Cost-effectiveness * Availability (Ravindran 2019) * Simplifies and automates bioanalytical techniques * High precision and sensitivity * Connectivity and computational power of smartphones. (Hernández-Neuta 2019 - doi: 10.1111/joim.12820)	* Absence of guidelines * Absence of a potential market for application as an identification tool (Ravindran 2019)	* Smartphones have been utilized either alone or combined with microscopy to detect and enumerate STH ova in endemic areas and resource limited settings (Ravindran 2019)	* Validity and feasibility: further focus is to be laid on validating these platforms and assessing their feasibility in clinical settings. (Hernández-Neuta 2019 - doi: 10.1111/joim.12820)	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low | Medium | High

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Link to SWOT draft

SWOT – EMERGING METHODS (6)

Method	Description	Strengths	Weaknesses	Opportunities	Threats	Color coding
Paper-Based Sensors	Paper microfluidics is a user friendly, low-cost technology, using paper as the solid matrix for managing the fluids in complex networks for identification of nucleic acid targets. (Magro 2017 - doi: 10.1038/s41598-017-00758-9)	* Adsorption * Excellent capillary action * Compatibility with environmental samples * Sterilization and disposal * The capability for the storage and transportation of reagents in the paper matrix * Lightweight and availability * Low cost * Simplicity (Ravindran 2019)	* Limitations in accuracy * Limitations in sensitivity * Inability to simultaneously detect more than one pathogen exist (Ravindran 2019)	* Routinely performed for the detection of pathogens * Detection of STH ova remains unexplored (Ravindran 2019)	* Access: although there are many proposals in the literature to develop NAATs in point-of-care (POC) devices, the access of the population to NAAT diagnostics still raises challenging issues in terms of cost, consumable availability, transportability, sample preparation and simplicity of the operation mode (Magro 2017 - doi: 10.1038/s41598-017-00758-9)	**Technical Feasibility** **Operational Feasibility** **Integration** **Cost**

Low | Medium | High

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Link to SWOT draft